**Effects of Perfluorohexan Vapor on Gas Exchange, Respiratory Mechanics, and Lung Histology in Pigs with Lung Injury after Endotoxin Infusion**

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**Background:** Inhaled perfluorohexan vapor has been shown to improve gas exchange and pulmonary mechanics in oleic acid– and ventilator-induced lung injury. However, in the clinical setting, lung injury frequently occurs in the context of systemic inflammation and consecutive lung injury, which may be induced experimentally by intravenous administration of endotoxin. The authors studied whether vaporized perfluorohexan is efficacious during endotoxin-induced lung injury in domestic pigs.

**Methods:** Twenty-two pigs (29 [23, 31] kg body weight [first, third interquartile]; tracheostomy) were anesthetized and mechanically ventilated. In the endotoxin (n = 8) and perfluorohexan groups (n = 7), we administered endotoxin of Escherichia coli 111:B4, 1 mg · kg⁻¹ · h⁻¹ for 1 h and 10 μg · kg⁻¹ · h⁻¹ for 5 h in consecutive order. In the perfluorohexan group, inhalation of the test drug was started 2 h 30 min after the start of the intravenous endotoxin and terminated after 30 min. In a control group (n = 7), animals were instrumented and observed over time without further intervention. Oxygenation function was assessed from oxygen partial pressures (P_{O_2}, blood gases) and calculated shunt fraction. Respiratory compliance was calculated from airway pressure and tidal volume. Measurements were performed before and every hour during endotoxin infusion.

**Results:** After 6 h of endotoxin, gas exchange and pulmonary compliance were deteriorated in the endotoxin group (P_{O_2}: 184 [111, 289] mmHg; 63 [45, 85], 58 [45, 70], mmHg, pulmonary shunt fraction: 42 [11.25, 38%], P < 0.05, endotoxin vs. control). Inhalation of vaporized perfluorohexan did not improve P_{O_2} (107 [60, 221] mmHg), pulmonary shunt fraction (32 [26, 58%]), or respiratory compliance (14 [10, 17] ml/mbar) when compared with intravenously administered endotoxin (not significant, perfluorohexan vs. endotoxin).

**Conclusions:** Inhalation of vaporized perfluorohexan does not improve pulmonary gas exchange or respiratory compliance in endotoxin-induced porcine lung injury.

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Materials and Methods

Study Design
The study was approved by the Bavarian Government and the Institutional Review Board for the care of animal subjects (both Munich, Germany). It was conducted in 22 pigs (German landrace; body weight 29 [23, 31] kg [first, third interquartile]), which were treated and housed in accordance with the principles of laboratory animal care.20

Anesthesia
After the pigs were fasted for one night with free access to water, intramuscular premedication was performed with midazolam (1 mg/kg) and ketamine (10 mg/kg). Anesthesia was induced by intravenous injection of fentanyl (20 μg/kg) and propofol (3 mg/kg), and muscular paralysis was induced by a single dose of atracurium besilate (0.7 mg/kg). All animals were placed in supine position and endotracheally intubated. Total intravenous anesthesia was maintained by continuous intravenous infusion of propofol (10 mg · kg⁻¹ · h⁻¹), midazolam (1.5 mg · kg⁻¹ · h⁻¹), and fentanyl (45 μg · kg⁻¹ · h⁻¹). No continuous relaxation was given. Normothermia was ensured by means of a warming pad and a warming lamp. Typically, long-term stabilization of gas exchange in swine subjected to the supine position requires aggressive mechanical ventilatory support, e.g., using positive end-expiratory pressure. Pilot experiments revealed that high end-expiratory pressures were not tolerated hemodynamically after endotoxin infusion. We decided to accept high tidal volumes as necessary to achieve stable gas exchange in the control group over 12 h (airway pressures of 28 [25, 30] / 11 [9, 12] / 5 [4, 5] at peak / mean / end-expiration; controlled minute ventilation at a fractional inspiratory oxygen concentration of 1.0, Servo 900 B; Siemens, Solna, Sweden). Based on repeated blood gas analyses, minute ventilation was adjusted to preserve normocapnia (Table 1). Because of the large surgical wound area and because of the lack of a ventilatory circuit heating, fluid losses required intravenous fluid replacement by Ringer’s solution (15 ml · kg⁻¹ · h⁻¹). Additional fluid consisting to equal parts of Ringer’s solution and pentastarch (6% hydroxyethyl starch 200,000/0.5; Fresenius-Kabi, Bad Homburg, Germany) was given as necessary to keep left ventricular preload (end-diastolic pressure 8 mmHg) constant. Intravenous norepinephrine was given continuously as necessary to maintain arterial pressure at 70 mmHg.

Surgical Preparation and Instrumentation
To minimize the surface of the artificial airway and to enable effective inhalation of drug aerosols, we performed tracheotomy in all animals. For collection of arterial and mixed venous blood samples, a femoral arterial introducer sheath (8.5 French; Arrow, Irvine, CA) and a fast-response thermodilution pulmonary artery catheter (7.5 French, Ref-1; Baxter, Irvine, CA) were inserted. For pressure recordings, an aortic and a left ventricular tip-manometer catheter (7 French, conduc-
tance tip; Millar Instruments, Houston, TX) were placed. A central venous catheter (14 gauge; Arrow; vena femoralis) allowed for continuous drug administration. Correct catheter positions were verified by fluoroscopy. Median sternotomy was performed, and an ultrasound

Table 1. Pulmonary Gas Exchange and Lactate Concentration during the Course of the Protocol

<table>
<thead>
<tr>
<th>Time Point of Measurement</th>
<th>Baseline</th>
<th>2 h after Endotoxin</th>
<th>4 h after Endotoxin</th>
<th>6 h after Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorohexan*</td>
<td>605 (575, 620)</td>
<td>299 (255, 356)†</td>
<td>263 (182, 297)†</td>
<td>107 (60, 221)†</td>
</tr>
<tr>
<td>Endotoxin*</td>
<td>572 (548, 600)</td>
<td>338 (257, 383)‡</td>
<td>289 (173, 451)‡</td>
<td>184 (114, 289)‡</td>
</tr>
<tr>
<td>Control</td>
<td>589 (574, 636)</td>
<td>574 (548, 610)</td>
<td>611 (582, 618)</td>
<td>638 (615, 658)</td>
</tr>
<tr>
<td>Lactate concentration, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorohexan*</td>
<td>1.1 (1.0, 1.4)</td>
<td>1.8 (1.6, 1.9)†</td>
<td>3.3 (2.7, 4.0)†</td>
<td>8.0 (2.6, 8.2)†</td>
</tr>
<tr>
<td>Endotoxin*</td>
<td>1.0 (0.9, 1.4)</td>
<td>2.3 (2.1, 2.6)‡</td>
<td>4.4 (3.3, 5.9)‡</td>
<td>7.5 (5.9, 12.1)‡</td>
</tr>
<tr>
<td>Control</td>
<td>1.2 (1.1, 1.6)</td>
<td>1.3 (0.8, 2.2)</td>
<td>1.6 (0.8, 2.2)</td>
<td>1.4 (0.8, 1.7)</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorohexan</td>
<td>31 (26, 33)</td>
<td>44 (36, 45)†</td>
<td>44 (41, 49)†</td>
<td>48 (46, 67)†</td>
</tr>
<tr>
<td>Endotoxin*</td>
<td>32 (30, 36)</td>
<td>42 (40, 47)‡</td>
<td>47 (44, 50)‡</td>
<td>66 (52, 70)‡</td>
</tr>
<tr>
<td>Control</td>
<td>30 (26, 35)</td>
<td>29 (26, 32)</td>
<td>28 (24, 32)</td>
<td>30 (25, 32)</td>
</tr>
</tbody>
</table>

Data are presented as median (first, third interquartile). Time points of measurement 1, 3, and 5 h after endotoxin are not given.

All parameters as presented were tested with a Friedmann test for changes over time in the groups (* P < 0.05). Differences between groups at the referring time points of measurement were tested with a Kruskal-Wallis test followed by a Student-Newman-Keuls test († P < 0.05, perfluorohexan vs. control; ‡ P < 0.05, endotoxin vs. control.

Lactate concentration = arterial lactate; PaO₂ = arterial oxygen partial pressure; PaCO₂ = arterial carbon dioxide partial pressure.
flow probe was placed around the pulmonary artery (14 mm; TC 208; Transonic Systems, Ithaca, NY). A urinary catheter was inserted surgically.

**Interventions**

After the surgical preparation, the animals were allowed to recover during a stabilization period of 30 min. A first set of control measurements was obtained thereafter. We then administered endotoxin of *Escherichia coli* 111:B4, 1 mg · kg⁻¹ · h⁻¹ for 1 h and 10 μg · kg⁻¹ · h⁻¹ for 5 h in consecutive order. Acute lung injury was assumed to be present when the clinical criteria as defined by the American and European Consensus Conference were fulfilled. After randomization using closed envelopes, the administration of the test drug was started 2 h 30 min after the start of the endotoxin infusion and terminated after a period of 30 min.

**Perfluorohexan Administration**

Perfluorohexan (C₆F₁₄, purity 95%; ABCR Chemie, Karlsruhe, Germany) is a clear fluid with a boiling point of 57°C, a vapor pressure of 177 mmHg at 20°C, a density of 1.672 g/ml, a viscosity of 0.66 cP, and a surface tension of 11.4 dyn/cm. The O₂ carrying capacity is 57 ml O₂ per 100 ml perfluorohexan (57 vol%). To administer perfluorohexan, we used a SIEMENS Servo B ventilator together with a modified vaporizer (isoflurane vaporizer model 952; SIEMENS Elema, Solna, Sweden) according to the method reported by Hubler et al. ²¹ By nature, the admixture of perfluorohexan vapor to the inspiratory gas reduces fractional inspiratory oxygen concentration. We therefore adjusted the respiratory volume during perfluorohexan inhalation. Expiratory partial pressure of carbon dioxide (Pco₂) was kept constant during inhalation.

**Verification of Drug Delivery**

To quantify the amount of vapor delivery, the inspiratory and expiratory concentrations of perfluorohexan were measured with near infrared spectroscopy on a modified infrared rapidly identifying analyzer for inhalation anesthetics (infrared rapidly identifying analyzer detector, PM8050; Dräger-Werke, Lubeck, Germany). The analyzer works on an infrared spectrum of 1–50 μm using a side stream of 200 ml/min. Calibration with gas chromatography determination gives a linear calibration curve for a signal generated by perfluorohexan on the enflurane detection chamber of the infrared rapidly identifying analyzer device, which is y = 1.6 x – 0.6; r² = 0.99 (Maximilian Ragaller, M.D., Ph.D., Department of Anesthesiology, Carl Gustav Carus University, Dresden, Germany, January 2002, written personal communication). To give evidence for successful drug delivery, we simultaneously administered (1) ambient air, (2) vaporized perfluorohexan, and (3) liquid perfluorohexan (300 ml) to two selectively intubated, ventilated *ex vivo* lung preparations. A three-dimensional data set, covering all three ventilated lung halves, was acquired with a single helical computed tomography scan (0.75 collimation, 80 mA, 456 KV) using a multidetector computed tomography scanner (Sensation 16; Siemens, Erlangen, Germany). Continuous axial 1-mm-thick images were reconstructed from the raw data. In the three-dimensional data set, a voxel represents a three-dimensional cuboidal area that is calculated out of the 1-mm slices. Density in every single voxel given in Hounsfield units served for further analysis. Before statistical analysis, all nonpulmonary voxels were removed from the image data with a semiautomatic threshold based method, leaving 366, 466 single voxels from the lung receiving partial liquid ventilation, 450, 280 voxels from the vaporized lung, and 450, 280 voxels from the control lung for analysis of density. ²² Histograms were calculated for each lung half separately, using Statistical Parametric Mapping software (SPM2/Matlab 13; MathWorks Inc., Natick, MA). ²³ Finally, direct detection of perfluorohexan in lung tissue was accomplished after exhaustive extraction of finely cut lung tissue using chloroform as an extracting agent. In the resulting solution, perfluorohexan could be detected qualitatively by ¹⁹F fluorine nuclear magnetic resonance spectroscopy. ²⁴–²⁶ This method is defined by an agitation signal that specifically detects ¹⁹F fluorine instead of hydrogen. All spectra were recorded on a spectrometer (Varian Inova 400 MHz; Varian GmbH, Darmstadt, Germany) using 1, 024 repeated measurements of nuclear resonance after agitation of the nuclei in an electromagnetic field at room temperature.

**Measurements**

Measurements were performed at baseline and with every further hour, beginning 1 h after the start of the endotoxin infusion, and included the parameters listed in the following sections.

**Hemodynamics.** Pulmonary arterial blood pressure was measured by means of a fluid filled pulmonary artery flotation catheter (REF-1 Ejection Fraction/Cardiac Output Computer; Baxter, Santa Ana, CA) and a Statham pressure transducer. Cardiac output was measured beat by beat by means of the ultrasound transit time technique ²⁷ with the pulmonary artery flow probe and indexed to body surface area (see appendix). ²⁸ Aortic pressure and left ventricular end-diastolic pressure were recorded online at 250 Hz (DasyLab; Measx, Mönchengladbach, Germany) with the tip-manometer catheters; all values were averaged over 20 s. Central venous pressure was measured *via* the proximal lumen of the pulmonary artery flotation catheter.

**Blood Samples.** Arterial and mixed venous blood samples were taken simultaneously in air-free syringes, cooled and analyzed instantly in duplicate; partial pressure of oxygen (P O₂) and Pco₂ were measured with a
blood gas analyzer (Chiron Diagnostics, Fernwald, Germany). Hemoglobin concentration and hemoglobin-oxygen saturation were measured by absorbance spectrophotometry on six different wavelengths (682 CO-oximeter; Instrumentation Laboratory, Lexington, MA).

**Tissue Samples.** Tissue samples were taken 6 h after start of endotoxin in duplicate from the left inferior lobe after ex vivo perfusion of the inflated lungs in situ with 10% formaldehyde through the pulmonary artery. The specimens were embedded in paraffin wax and cut into cross-sections of 5 μm on a microtome. After staining with hematoxylin and eosin, the slices were examined by light microscopy by a pathologist who was not aware of the treatment group. Lung injury was scored from 0 (no damage) to 5 (maximal damage) according to combined assessments of alveolar hemorrhage and edema, infiltration of neutrophils in airspace or vessel walls, and hyaline membranes.29 To visualize the infiltration of lung tissue with neutrophils, on paraffin-embedded slices of 10 μm, histochemical chloracetatesterase staining was performed. The total number of stained cells in 50 visual fields on a magnification of 100× was determined. The examination was performed with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

**Calculations**
Systemic and pulmonary vascular resistance, pulmonary shunt fraction, and respiratory compliance were calculated using standard formulae. Data were indexed to body surface area, where appropriate. The referring formulae are listed in the appendix.

**Statistical Analysis**
Physiologic data were tested for normal distribution with a Shapiro-Wilks test. Because most of the data were not normally distributed, they are presented as median (first, third interquartile). Differences of measured values over time were tested with a Friedman repeated-measurement analysis of variance on ranks (seven time points of measurement) or a signed-rank test (two time points of measurement). Differences of the parameters between the perfluorohexan, endotoxin, and control groups were analyzed with a Kruskal-Wallis test, followed by a Student-Newman-Keuls test (The SAS-System, release 8.2; The SAS Institute, Cary, CA; automatic correction for repeated testing). Computed tomography data were analyzed for normality of voxel density (Kolmogorov-Smirnov test). Normality was given in all three samples. Thus, differences in mean voxel density (Hounsfield units) were tested with an independent t test. Testing for differences in the density distribution in the 366, 466 voxels from the partially liquid ventilated lung, in the 450, 280 voxels from the vaporized lung, and in the 450, 280 voxels from the lung receiving ambient air was performed using a Kolmogorov-Smirnov test. To enable investigation of differences in distribution of values unaffected by coexistent differences in means, the referring histograms were centered, i.e., every single value was corrected by the mean difference between both voxel histograms (Statistica 5.1; StatSoft, Tulsa, OK, and SPSS 12.0; SPSS Inc., Munich, Germany).

**Results**

**Data Acquisition, Stability of the Model, Drug Delivery**
At baseline, values displayed no differences between groups. Despite a slight increase in mean pulmonary arterial pressure, peak airway pressure and peripheral leukocyte count, all parameters remained unchanged over the 6-h period in the control group (tables 1–3). In 15 animals, endotoxin was administered and a complete data set was obtained. In 7 animals, perfluorohexan was inhaled. Systemic inflammation was induced in all animals receiving intravenous endotoxin, as documented by increased fluid and catecholamine requirements to maintain preload and peripheral resistance stable. At 6 h, cumulative fluid requirements to keep left ventricular end-diastolic pressure at 8 mmHg were 2, 000 ml in the perfluorohexan group and 1, 810 ml in the endotoxin group as compared with 600 ml in the control group (P < 0.05 vs. perfluorohexan, vs. endotoxin; table 2). The mean turnover of the vaporizer was 750–1, 000 ml in 30 min. Inspiratory concentration of perfluorohexan reached 18 vol%. Ex vivo computed tomography scans of lung preparations evidence distal distribution of perfluorohexan vapor in the lungs (~823 vs. ~867 Hounsfield units; P < 0.001; fig. 1) and 19fluorine-nuclear magnetic resonance spectroscopy evidenced the presence of perfluorohexan in peripheral lung tissue.

**Pulmonary Tissue Damage**
Ventilator-induced alterations of lung tissue and leukocyte sequestration were mild in the control group. We observed a massive invasion of neutrophils and significant alteration of alveolar integrity in the endotoxin and perfluorohexan groups (see examples in figs. 2A and B).

**Gas Exchange**
The parameters of gas exchange (PaO2, pulmonary shunt fraction) remained unchanged in the control group throughout the whole observational period (fig. 3A and table 1). In the endotoxin and perfluorohexan groups, gas exchange dramatically deteriorated at 2 h, fulfilling clinical criteria of acute lung injury at 5 h, and criteria of acute respiratory distress syndrome at 6 h in the endotoxin group (P < 0.05 over time, endotoxin vs. control; table 1). In the perfluorohexan group, acute lung injury was established at 4 h and acute respiratory distress syndrome was established at 6 h after start of endotoxin. Inhaled perfluorohexan had no effect on gas
exchange at any point in time when compared with endotoxin alone (not significant, perfluorohexan vs. endotoxin; \( P < 0.05 \), perfluorohexan vs. control; fig. 3A and table 1).

Pulmonary Mechanics

Calculated respiratory compliance remained unchanged in the control group (not significant over time). In the endotoxin group, respiratory compliance was

Table 2. Vasopressor Support, Cardiac Preload, and Systemic Blood Pressure during the Course of the Protocol

<table>
<thead>
<tr>
<th>Time Point of Measurement</th>
<th>Norepinephrine, mg/h</th>
<th>MAP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>MPAP, mmHg</th>
<th>CVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perfluorohexan*</td>
<td>Endotoxin*</td>
<td>Control</td>
<td>Perfluorohexan*</td>
<td>Endotoxin*</td>
</tr>
<tr>
<td></td>
<td>0.4 (0.4, 0.8)†</td>
<td>1.0 (0.6, 1.6)†</td>
<td>0.6 (0.4, 3.3)†</td>
<td>0.6 (0.4, 3.3)†</td>
<td>1.5 (0.7, 3.8)†</td>
</tr>
<tr>
<td>2 h after Endotoxin</td>
<td>66 (62, 73)</td>
<td>70 (67, 71)</td>
<td>79 (72, 97)</td>
<td>8.0 (8.0, 8.5)</td>
<td>45 (41, 47)†</td>
</tr>
<tr>
<td>4 h after Endotoxin</td>
<td>67 (64, 78)</td>
<td>61 (57, 69)</td>
<td>75 (74, 84)</td>
<td>8.0 (8.0, 8.5)</td>
<td>45 (41, 47)†</td>
</tr>
<tr>
<td>6 h after Endotoxin</td>
<td>62 (62, 65)</td>
<td>67 (60, 74)</td>
<td>70 (68, 75)</td>
<td>8.0 (8.0, 8.5)</td>
<td>49 (44, 50)†</td>
</tr>
</tbody>
</table>

Data are presented as median (first, third interquartile). Time points of measurement 1, 3, and 5 h after endotoxin are not given.

All parameters as presented were tested with a Friedman test for changes over time in the groups (* \( P < 0.05 \)). Differences between groups at the referring time points of measurement were tested with a Kruskal-Wallis test followed by a Student-Newman-Keuls test († \( P < 0.05 \), perfluorohexan vs. control; ‡ \( P < 0.05 \), endotoxin vs. control.

CVP – central venous pressure; LVEDP – left ventricular end-diastolic pressure; MAP – mean arterial pressure; MPAP – pulmonary arterial pressure.

Table 3. Cardiac Output, Heart Rate, and Vascular Resistance during the Course of the Protocol

<table>
<thead>
<tr>
<th>Time Point of Measurement</th>
<th>CI, l/min ( \cdot ) m ( ^{-2} )</th>
<th>HR, min ( ^{-1} )</th>
<th>SVRI, dyn ( \cdot ) s ( \cdot ) cm ( ^{-5} ) ( \cdot ) m ( ^{-2} )</th>
<th>PVRI, dyn ( \cdot ) s ( \cdot ) cm ( ^{-5} ) ( \cdot ) m ( ^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perfluorohexan*</td>
<td>Endotoxin*</td>
<td>Control</td>
<td>Perfluorohexan*</td>
</tr>
<tr>
<td></td>
<td>4.2 (3.8, 4.5)</td>
<td>4.4 (3.8, 5.0)</td>
<td>3.4 (3.1, 3.8)</td>
<td>85 (76, 92)</td>
</tr>
<tr>
<td>2 h after Endotoxin</td>
<td>4.1 (3.7, 5.7)†</td>
<td>5.4 (4.9, 6.6)†</td>
<td>3.5 (3.2, 3.8)</td>
<td>88 (84, 96)</td>
</tr>
<tr>
<td>4 h after Endotoxin</td>
<td>5.1 (4.4, 5.6)†</td>
<td>4.8 (4.3, 5.9)†</td>
<td>3.2 (3.0, 3.4)</td>
<td>94 (90, 103)</td>
</tr>
<tr>
<td>6 h after Endotoxin</td>
<td>3.2 (2.7, 3.9)</td>
<td>3.5 (2.8, 5.3)</td>
<td>3.0 (2.6, 3.2)</td>
<td>96 (92, 121)†</td>
</tr>
</tbody>
</table>

Data are presented as median (first, third interquartile). Time points of measurement 1, 3, and 5 h after endotoxin are not given.

All parameters as presented were tested with a Friedman test for changes over time in the groups (* \( P < 0.05 \)). Differences between groups at the referring time points of measurement were tested with a Kruskal-Wallis test followed by a Student-Newman-Keuls test († \( P < 0.05 \), perfluorohexan vs. control; ‡ \( P < 0.05 \), endotoxin vs. control.

CI – cardiac index; HR – heart rate; PVRI – pulmonary vascular resistance index; SVRI – systemic vascular resistance index.

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reduced at 3, 4, 5, and 6 h after start of endotoxin ($P < 0.05$ over time, endotoxin vs. control; fig. 3B). Inhaled perfluorohexan had no effect on compliance when compared with intravenous endotoxin at the investigated time points of measurement (not significant, perfluorohexan vs. endotoxin; $P < 0.05$, perfluorohexan vs. control; fig. 3B).

Discussion

The results of the current investigation indicate that perfluorohexan vapor, inhaled in a concentration of 18 vol%, has no effects on the degree of pulmonary tissue damage, on gas exchange, or on pulmonary mechanics in endotoxin-induced acute lung injury.

This is surprising because inhalation of vaporized perfluorohexan in a concentration of 18 vol% improved PaO$_2$ and pulmonary shunt fraction when administered in oleic acid–induced acute lung injury in sheep, where perfluorohexan led to a long-lasting improvement of oxygenation.\textsuperscript{14} Although the drainage of perfluorocarbons from the lung after termination of partial liquid ventilation is known to induce a marked deterioration of gas exchange in canine acute lung injury, the effect of vaporized perfluorohexan in the study by Bleyl \textit{et al.}\textsuperscript{14} persisted over 2 h after perfluorohexan had been withdrawn. Likewise, the reduction of peak airway pressure persisted for 2 h after the inhalation of perfluorohexan.

Fig. 1. Six typical examples of computed tomography scans from three nonperfused, \textit{ex vivo} ventilated lungs. Half of the figures show raw scans in two-dimensional technique. Half show the corresponding calculated three-dimensional reconstructions consisting of calculated voxels. The computed tomography scan was performed during partial liquid ventilation with perfluorohexan (liquid ventilation, 300 ml), during inhalation of perfluorohexan vapor (vapor inhalation), and during mechanical ventilation using room air (control). The three histograms in the plot represent the frequency distribution of density given in Hounsfield units within each of the three voxel data sets out of each lung preparation. Lung density is depicted in classes of 16 Hounsfield units on the x-axis. On the y-axis, frequency of values within every class is given. The mean density value during perfluorohexan vapor inhalation was increased as compared with room air ventilation ($\# P < 0.001$, test), suggesting the presence of perfluorohexan vapor. The distribution pattern (shape) during partial liquid ventilation was significantly wider than during vapor inhalation ($\# P < 0.01$, Kolmogorov-Smirnov test; see Materials and Methods), indicating more inhomogenous density distribution (high and low values were present). This probably relates to the presence of bullae and fluid-filled areas of the lung in the native computed tomography scans during partial liquid ventilation and was absent during vapor inhalation.
based on its low surface tension (11.4 dyn/cm). The recruitment of atelectatic lung regions may thus be enhanced by the high spreading factor of perfluorocarbons. In addition, perfluorocarbons have a high physical oxygen solubility (57 vol% in perfluorohexan) in liquid ventilation, and they have antioxidative properties. The fact that we did not observe any positive perfluorohexan-related effects in our study may relate to (1) wrong dosage, (2) the vaporization procedure itself, (3) an unsuccessful pulmonary perfluorohexan delivery, (4) the specific nature of the model, (5) the time point of administration, or (6) a lack of efficacy in sepsis-induced acute lung injury.

**Drug Dosage and Delivery**

In the current study, exactly the same dosages (18 vol%) were chosen that were reported in the literature.14,21,30 We used a SIEMENS Servo ventilator together with a modified vaporizer for Servo ventilators (isoflurane vaporizer model 952). This setup has been successfully used for inhalation of vaporized perfluorohexan in acute lung injury by Hubler et al.21 Based on the calibration from the cited work of Bleyl et al., we could evidence correct dosage obtained by near infrared spectroscopy (infrared rapidly identifying analyzer; Dräger PM 8050; see Materials and Methods, data not shown). Despite correct vaporization, theoretically, the possibility exists that no perfluorohexan has reached the site of action (terminal airways). However, in pilot animals, we were able to demonstrate that this was not the case. As depicted by computed tomography and as is known from the literature, inhalation of gases such as perfluorohexan leads to a homogenous distribution in the lung. In contrast to inhalation of perfluorohexan in the form of vapor or in the form of an aerosol, liquid ventilation is followed by a gravitationally governed, more inhomogenous distribution within the lung, which is displayed in the different shapes of histograms during partial liquid ventilation and vapor inhalation (different

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**Fig. 2.** A and B depict parameters illustrating the pulmonary tissue damage. A shows two examples of histologic changes as observed 6 h after start of endotoxin in the endotoxin group, the perfluorohexan group, and the control group, respectively. At a magnification of 200×, the left specimens show scarcely any leukocytes, clear alveolar spaces, and slim alveolar walls. The examples from the perfluorohexan and endotoxin groups on the right depict a massive sequestration of leukocytes into the alveolar walls, the accumulation of detritus in the alveolar spaces, and a thickening of alveolar walls. B shows lung fluid content expressed as wet-to dry ratio (quotient without dimension on the y-axis), quantified leukocyte sequestration as median of counted cells in 50 fields, and tissue damage as deduced from the semiquantitative histologic score. The control group is indicated by gray bars, the endotoxin group is indicated by black bars, and the perfluorohexan group is indicated by white bars. Differences between the groups are tested with a Kruskal-Wallis test, followed by a Student-Newman-Keuls test. # Significant difference between the endotoxin and control groups. § Significant difference between the perfluorohexan and control groups. All parameters of tissue damage were increased in the perfluorohexan and endotoxin groups as compared with the control group. The inhalation of perfluorohexan did not induce a reduction of the pulmonary injury. WBC = white blood cell.
density distribution in fig. 1; \( P < 0.01 \). From the \( ^{19} \)fluorine spectroscopy of sampled lung tissue, we conclude that perfluorohexane was successfully deposited during vapor inhalation. Furthermore, by \( ^{19} \)fluorine spectroscopy, we clearly identified the spectrum of our test drug to be perfluorohexane.

Experimental Model and Procedure

By nature, blinding was not feasible in the current study, leaving the theoretical possibility of investigator bias. To demonstrate stability of the model and to exclude that time-dependent changes might influence our results, a control group was implemented.

In the control group, we observed discrete histologic changes in the pulmonary tissue and a mild increase in pulmonary arterial pressure 6 h after the time when endotoxin was started in the other groups. These changes most likely relate to the surgical trauma, the ventilatory pattern, or both. A limitation of our study is that the tidal volumes administered to the animals (\(-20 \text{ ml/kg}\)) are higher than currently recommended (6 ml/kg). These high tidal volumes are not applicable in humans. We decided to preserve gas exchange not using high airway pressures, because in pilot experiments, high end-expiratory pressures interfered with hemodynamic stability after endotoxin. As displayed in the catecholamine and volume requirements of our data, a further hemodynamic insult would have interfered with the feasibility of our model. As a methodologic drawback, high tidal volumes as used in our model are known to exert ventilator-induced acute lung injury, and they might be responsible for the observed slight changes in histologic cross-sections and in pulmonary artery and airway pressure in the control group at 6 h. However, all other parameters reported herein (including \( \text{PaO}_2 \) and pulmonary shunt fraction) remained unchanged over time in the control group, suggesting that no ventilator-induced acute lung injury was present in our study. The infusion of endotoxin caused systemic inflammation in the endotoxin and perfluorohexane groups, as illustrated by increased fluid and catecholamine requirements. Acute lung injury was established in both treatment groups as documented by pronounced interstitial and alveolar damage. The findings include alveolar and interstitial edema, alveolar hemorrhage, and infiltration of inflammatory cells, mainly neutrophils. The morphologic injury qualitatively agrees with early acute lung injury in humans. Correspondingly, we observed the typical changes in gas exchange and pulmonary artery pressure.

Even if acute lung injury is interpreted as a combination of endotoxin-induced and superimposed ventilator-induced acute lung injury in our investigation, the stability of the model is depicted in our data set. Furthermore, the latter fact should not affect the results with respect to the efficacy of inhaled perfluorohexane vapor. In terms of a representative model design, one could argue that, in principle, in the clinical situation of systemic inflammation the treatment of lung injury with aggressive mechanical ventilation is not an uncommon mistake.

The type of lung injury chosen in our study might be the cause for the lack of efficacy of inhaled perfluoro-
hexan: First, in the early stage of acute lung injury, intravenous endotoxin elicits damage on the endothelial and interstitial side (see cross-sections). Second, the complete destruction of the alveolocapillary membrane requires hours, because it is mediated immunologically. One could speculate that these are the reasons why perfluorohexan being present in the terminal airways did not develop the full spectrum of its beneficial effects after endotoxin infusion in pigs.

**Implications of the Findings**

Our findings do not support previous studies demonstrating an increase in PatO2, an increase in compliance, and a decrease in pulmonary arterial pressure after inhalation of 18% perfluorohexan in oleic acid– and ventilator-induced acute lung injury. This suggests that perfluorohexan may only be effective in some conditions, where the lung has been directly affected, and not when it has been damaged during a more general injury. Clinically, lung injury by remote sepsis is frequent. Our data suggest that the effects of this type of injury may not respond to perfluorohexan vapor, and clinical assessment might be premature.

**Methods of Measurement**

We decided to determine the pulmonary sequestration of leukocytes from histologic cross-sections, because the injury was performed from the vascular side. Interstitial accumulation of activated cells might be expected before alveolar accumulation of cells. Fifty microscopic fields were counted by an experienced pathologist, and the median was calculated. The assessment of tissue damage from histologic cross-sections is, by nature, not quantitative. To enable independent assessment and comparison, all cross-sections were blinded and investigated, by a pathologist who was not aware of the treatment group, using a semiquantitative score.25 The measurement of lung wet-to-dry ratio was highly standardized. Drying of the lung tissue was extended to 21 days in all samples. To minimize the influence of hydrostatic effects on tissue water content, we always chose the lobus trachealis of the right lung. The tissue sample was excised exactly at the interlobular septum. Because the number of lungs investigated was relatively low (n = 4, 4, and 5), the analysis of variance did not reach statistical significance (P = 0.056), and no post hoc testing was performed.

**Conclusion**

Our data suggest that in contrast to our hypothesis, inhalation of vaporized perfluorohexan has no beneficial effect on pulmonary tissue damage, gas exchange, or pulmonary mechanics when performed in acute lung injury secondary to endotoxin-induced systemic inflammation in swine. Previous data showed notable positive effects of perfluorohexan on the extent of inflammation, on gas exchange, and on pulmonary mechanics in various other experimental models of lung injury. Therefore, efficacy of inhaled perfluorohexan in experimental lung injury may depend on the chosen model. Hence, our data suggest that further studies on the mechanisms of action are needed.

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**References**


Appendix

\[
\text{BSA (m}^2) = 10^3 \cdot \text{body wt (kg)}^{2/3} \cdot k \cdot 10^{-4}
\]

\[
\text{Cl (l \cdot min}^{-1} \cdot \text{m}^{-2}) = \text{CO (l/min) \cdot BSA (m}^2)^{-1}
\]

\[
\text{SVRI (dyn} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}) = \left[\frac{\text{MAP (mmHg) – CVP (mmHg)}}{79.9 \cdot \text{CO (l/min)}^{-1}}\right] \cdot \text{BSA (m}^2)^{-1}
\]

\[
\text{PVRI (dyn} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{m}^{-2}) = \left[\frac{\text{LVEDP (mmHg) – CVP (mmHg)}}{79.9 \cdot \text{CO (l/min)}^{-1}}\right] \cdot \text{BSA (m}^2)^{-1}
\]

\[
\text{CaO}_2 \text{ (ml/100 ml)} = 1.34 \cdot \text{[hemoglobin (g/dl)]} \cdot \text{SaO}_2 \% \cdot \left(100^{-1} + 0.0031 \cdot \text{Pao}_2 \text{ (mmHg)}\right)
\]

\[
\text{CcO}_2 \text{ (ml/100 ml)} = 1.34 \cdot \text{[hemoglobin (g/dl)]} \cdot \text{SvO}_2 \% \cdot \left(100^{-1} + 0.0031 \cdot \text{Pvo}_2 \text{ (mmHg)}\right)
\]

\[
\text{Pao}_2 \text{ (mmHg)} = \text{FiO}_2 \cdot \left(\text{pbaro (mmHg)} – \text{ph}_2O_{37°C} \text{ (mmHg)}\right) – \text{PaCO}_2 \text{ (mmHg)} / 0.8
\]

\[
\text{CcO}_2 \text{ (ml/100 ml)} = 1.34 \cdot \text{[hemoglobin (g/dl)]} + 0.0031 \cdot \text{Pao}_2 \text{ (mmHg)}
\]

\[
\text{VO}_2J \text{ (ml/min}^{-1} \cdot \text{m}^{-2}) = 10 \cdot \left(\frac{\text{CaO}_2 \text{ (ml/100 ml)} – \text{Cvo}_2 \text{ (ml/100 ml)}}{\text{Cl} (l \cdot \text{min}^{-1} \cdot \text{m}^{-2})} + \text{Cl} (l \cdot \text{min}^{-1} \cdot \text{m}^{-2})\right)
\]

\[
\text{Qo}/\text{QI} \% = \left(\frac{\text{CcO}_2 \text{ (ml/100 ml)} – \text{CoO}_2 \text{ (ml/100 ml)}}{\text{Cvo}_2 \text{ (ml/100 ml)}}\right) \cdot \text{Cl} (l \cdot \text{min}^{-1} \cdot \text{m}^{-2})
\]

\[
\text{C}_{\text{HS}} \text{ (ml/mbar)} = V_i (ml) \cdot \text{Paw_plat (mbar)}^{-1}
\]

body wt = body weight; BSA = body surface area; CaO2 = arterial oxygen content; CcO2 = pulmonary capillary oxygen content; Cl = cardiac index; CO = cardiac output; Cint = respiratory compliance, consisting of pulmonary and thoracic compliance; Cvo2 = mixed venous oxygen content; CVP = central venous pressure; DO2Jl = global O2 delivery; [Hb] = arterial hemoglobin concentration; k = species-specific body surface area constant, which is 9 for pigs; LVEDP = left ventricular end-diastolic pressure; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; MPAP* = arterial carbon dioxide partial pressure; PaO2 = arterial oxygen partial pressure; PaO2 = arterial oxygen partial pressure; Paw_plat = end-inspiratory airway pressure; pH2O37°C = water vapor pressure at a temperature of 37°C; Pvo2 = mixed venous oxygen partial pressure; PVRI = pulmonary vascular resistance; Qo/QI = pulmonary shunt fraction; SaO2 = arterial hemoglobin oxygen saturation; SVRI = systemic vascular resistance; SvO2 = mixed venous hemoglobin oxygen saturation; VO2J = global O2 consumption; Vi = respiratory tidal volume.

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